## **UTILITY PATENT APPLICATION TRANSMITTAL** (Small Entity)

1075.00022

Docket No.

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Total Pages in this Subr

### TO THE ASSISTANT COMMISSIONER FOR PATENTS Box Patent Application

Washington, D.C. 20231

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# UTILITY PATENT APPLICATION TRANSMITTAL (Small Entity)

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Docket No. 1075.00022

Total Pages in this Submission

### Application Elements (Continued)

3.	X	rawing(s) (when necessary as prescribed by 35 USC 113)										
	a.	□ Formal b. 🗷 Informal Number of Sheets 22										
4.	X	oath or Declaration										
	a.	Newly executed (original or copy)										
	b.	Copy from a prior application (37 CFR 1.63(d)) (for continuation/divisional application only)										
	C.	With Power of Attorney										
	d.	<u>DELETION OF INVENTOR(S)</u> Signed statement attached deleting inventor(s) named in the prior application, see 37 C.F.R. 1.63(d)(2) and 1.33(b).										
5.	X	Incorporation By Reference (usable if Box 4b is checked) The entire disclosure of the prior application, from which a copy of the oath or declaration is supplied under Box 4b, is considered as being part of the disclosure of the accompanying application and is hereby incorporated by reference therein.										
6.		omputer Program in Microfiche										
7.		enetic Sequence Submission (if applicable, all must be included)										
	a.	Paper Copy										
	b.	Computer Readable Copy										
	C.	Statement Verifying Identical Paper and Computer Readable Copy										
		Accompanying Application Parts										
8.		Assignment Papers (cover sheet & documents)										
9.		7 CFR 3.73(b) Statement (when there is an assignee)										
10.		nglish Translation Document (if applicable)										
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# UTILITY PATENT APPLICATION TRANSMITTAL (Small Entity)

Docket No. 1075.00022

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Total Pages in this Submission

			Ac	companying Ap	plication Pa	rts (Continued)				
15.		Certified C	opy of Priority	Document(s) (if i	foreign priorit	is claimed)				
16.	16. 🗷 Small Entity Statement(s) - Specify Number of Statements Submitted:									
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### Attorney's Docket Number:

Applicant or Patentee:	Nurith Shaklai								
Serial or Patent No:									
Filed or Issued:	Herewith								
For:	A METHOD FOR THE LONG-TERM PRESERVATION OF MEAT AND THE MEAT PROCESSED THEREBY								
VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS (37 CFR 1.9(f) and 1.27(d)SMALL BUSINESS CONCERN									
I hereby declare that I	am:								
the owner	of the small business concern identified below:								
X an offici behalf of	al of the small business concern empowered to act on the concern identified below:								
Name of Concern: R	AMOT-University Authority for Applied Research nd Industrial Development Ltd.								
	H. Levanon Street, P.O. Box 39296								
I	el Aviv 61392 ISRAEL								
I hereby declare that the above identified small business concern qualifies as a small business concern as defined in 13 CFR 121.3-18, and reproduced in 37 CFR 19.9(d), for purposes of paying reduced fees under Section 41(a) and (b) of Title 35. United States Code, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement: (1) the number of employees of the business concern is the average over the previous fiscal year of the concern of the persons employed on a full-time, part-time or temporary basis during each of the pay periods of the fiscal year and (2) concerns are affiliates of each other when, either directly or indirectly, one concern controls or has the power to control the other, or a third-party or parties controls or has the power to control both.  I hereby declare that rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the									
<pre>invention entitled: By Inventor(s):</pre>									
Described in:									
	cation filed herewith.								
	n serial no, filed								
patent no.	, issued								
(Small Ent	ity-Small Business [Form 7-4]Page 1 of 2)								

If the rights held by the above-identified small business concern are not exclusive, each individual, concern or organization having rights to the invention is listed below and no rights to the invention are held by any person, other than the inventor, who would not qualify as an independent inventor under 37 CFR 1,9(c), if that person made the invention, or by any concern which would not qualify as a small business concern under 37 CFR 1,9(d) or a nonprofit organization under 37 CFR 1,9(d)

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(Small Entity-Small Business (Form 7-4) -- Page 2 of 2)

### IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: NURITH SHAKLAL

Continuation-In-Part of Divisional Patent Application of

Serial No.: 09/480,812

Filed: herewith

For: A METHOD FOR THE LONG-TERM PRESERVATION OF MEAT AND THE MEAT PROCESSED THEREBY

Attorney Docket No..: 1075.00022

### PRELIMINARY AMENDMENT

Assistant Commissioner for Patents Washington, D.C. 20231 Box Patent Application Sir:

Please preliminarily amend the above-captioned Continuation-In-Part patent application prior to examination as follows:

### IN THE SPECIFICATION:

Page 1, in the "Cross-Reference to Related Application Section", after "This is a", please insert:

—Continuation-In-Part application of United States Patent Application Serial No. 09/480,812, filed January 10, 2000, which is a divisional of United States Patent Application Serial No. 08/945,264, filed January 20, 1998, now United States Patent No. 6,042,859, all of which are incorporated herein by reference—.

Page 1, line 3, after "the present invention relates to a method for preserving raw meat", please insert the following: —and preventing micro growth—

Page 4, line 21, after "refrigeration" please insert the following:

-Generally, two classes of microorganisms are considered to be undesirable. The first class being aerobic and the second class being anaerobic. The anaerobic microorganisms include facultative (having both aerobic and anaerobic metabolic pathways). The aerobic microorganisms cannot survive without oxygen while the anaerobic microorganisms have a non-oxygen requirement for metabolism. It is important to note that iron is an essential element for both organisms.

For both, growth of microorganisms spoil the meat by changing texture, appearance and the development of bad odors. The pathogenic bacteria are those interfering with the human host metabolism and interfere with normal physiology causing effects ranging from the minor to the lethal. The aerobic bacteria are likely to arrive from outside of the host organism. This is in contradistinction from the anaerobic facultative microbes which may be animal borne because they can grow in the host devoid of free oxygen. However, neither bacteria are desirable to have on the meat. It is therefore important to develop ways of eliminating bacterial growth be it anaerobic or aerobic. —

Page 10, line 12, please insert after "quality." —Also provided by the present invention is a method of preventing microorganism growth in meat by exposing raw meat to an atmosphere consisting essentially of carbon monoxide.

Page 12, line 24, please delete "and".

Page 13, line, please delete "." and insert therefor --;--.

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Page 13, after Figure 13, please insert the following:

--Figure 14 is a graph showing the growth of E coli on beef under a defined atmosphere having either untreated or high carbon monoxide atmospheres;

Figure 15 is a graph showing the growth of *E coli* and beef under defined atmosphere having either untreated or low carbon monoxide atmosphere:

Figure 16 is a graph showing the growth of Pseudomonas fluorescence on beef under defined atmospheres having untreated atmosphere, low carbon monoxide or high carbon monoxide atmosphere;

Figure 17 is a graph showing the growth of Staphylococcus aures on beef under a defined atmospheres, the atmosphere either being untreated or with high carbon monoxide;

Figure 18 is a graph showing the growth of Listeria monocytogenes on beef under a defined atmospheres, the atmosphere is being either untreated, low carbon monoxide, or high carbon monoxide;

Figure 19 is a graph showing the growth of Clostridium prefringens on beef under defined atmospheres as having either untreated, low carbon monoxide or high carbon monoxide atmospheres;

Figure 20 is a graph showing the growth of Salmonella Typhimirium on beef under defined atmospheres, either untreated, low carbon monoxide or high carbon monoxide atmospheres;

Figure 21 is a graph showing the growth of Listeria monocytogenes on poultry under defined atmospheres of either untreated or high carbon monoxide atmospheres;

Figure 22 is a graph showing the growth of Pseudomonas fluorescence on poultry under defined atmospheres of either untreated atmosphere or high carbon monoxide atmosphere; and

Figure 23 is a graph showing the growth of clostridium prefringens on poultry under defined atmospheres of either untreated, low carbon monoxide or high carbon monoxide atmosphere.—

Page 13, line 15, after "container," please insert the following:

--The present invention also provides a method for preventing microorganism growth on preserved meat by exposing raw meat, processed or not, to an atmosphere consisting essentially of carbon monoxide, and subsequently storing the meat in a sealed container.--

Page 15, line 6, please insert:

--The term "microorganisms" is defined as being either aerobic or anaerobic organisms which can survive on meat. These can include, but are not limited to, *E coli* Pseudomonas fluorescence, Staphylococcus aures, Listeria monocytogenes. Clostridium prefringens, and Salmonella Typhimirium.--

Page 16, line 26, please insert:

--The data included herewith have established that the growth of all pathogens was inhibited when meat was treated under a carbon monoxide containing atmosphere. Further, the level of inhibition increases upon the elevation of the carbon monoxide content in the modified atmosphere. Therefore, by exposing meat to an atmosphere containing carbon monoxide, not

only is the meat itself preserved but further, there is an inhibition of pathogen growth on the meat. This inhibition enables the life span of the meat to be increased without sacrificing any safety in distributing the meat to be consumed.

### Page 46, line 1, please insert the following:

### --Example 14

Studies were carried out comparing growth of inoculated pathogenic bacteria on fresh meat under three atmospheres. Air and two CO containing modified atmospheres: high CO (95-100%) and low (10%) CO. In low CO MA CO was replaced by an inert gas, nitrogen. Note that in most experiments the CO untreated control was the same for both modified atmospheres. Experiments with *E coli* were carried out on different meat controls.

### Conclusions

The data indicate that none of the anaerobic/facultative pathogens packed under CO atmosphere could grow better than when packed under air or nitrogen. In contrast, growth of all pathogens was inhibited under CO containing atmosphere and the level of inhibition was stronger upon elevation of CO content in the modified atmosphere.

As shown in Figures 14-23, the methods of the present invention inhibit the growth of *E coli*, Pseudomonas Staphylococcus aures Listeria monocytogenes, clostridium prefringens Salmonella Typhimirium. This effect was shown in both beef and in poultry, thus showing that this effect is not limited to use in the beef industry, but can instead be used in other similar industries wherein the growth of micro is undesirable.--

### IN THE CLAIMS:

[A method for preserving meat by:] A method of inhibiting pathogen growth by:

exposing raw meat to an atmosphere consisting essentially of carbon monoxide and maintaining the meat in a vacuum free, sealed container to maintain color and freshness while retarding bacterial growth.

Please cancel claims 14-30

- 31. (New) An inhibitor of pathogen growth on meat comprising an atmosphere of carbon monoxide.
- 32. (New) The inhibitor as set forth in claim 31, wherein said atmosphere is further defined as a volume of carbon monoxide of approximately 30% by weight or volume of the meat being treated.

### REMARKS

Claims 1-13 and 31-32 remain in the application.

The application is now in condition for allowance, which allowance is respectfully solicited.

Respectfully submitted,

KOHN & ASSOCIATES

Amy E. Rinaldo Registration No. 45,791

30500 Northwestern Highway Suite 410

Farmington Hills, MI 48334

(248) 539-5050

# SUPPLIES BULLES

### CERTIFICATE OF MAILING BY "EXPRESS MAIL"

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Washington, DC 20231.

Connie Herty

### A METHOD FOR THE LONG-TERM PRESERVATION OF MEAT AND THE MEAT PROCESS THEREBY

### BACKGROUND OF THE INVENTION

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### TECHNICAL FIELD

The present invention relates to a method for preserving raw meat and more specifically relates to a method for preserving raw meat by exposing the meat to an atmosphere consisting essentially of carbon monoxide.

### BACKGROUND ART

It is well known in the meat processing

industry that from the time animals are slaughtered, measures must be taken to preserve the meat and prevent it from becoming rancid or spoiled. The measures to preserve raw meat must be implemented and carried through from the time the animal is first slaughtered through the time the meat is purchased and ultimately consumed by the purchaser.

Historically, preservation of the freshness or quality of the meats has been practiced for hundreds, if not thousands of years.

Early preservation techniques of meat took the form of drying or "jerking" meat and packing or storing

cuts of meat in salt. This method, while somewhat effective for preserving meat and keeping it from becoming spoiled, had many drawbacks not the least of which was the incorporation of large amounts of salt into meat slated for human consumption.

The use of additives or preservatives such as nitrates and nitrites to meats is another common technique for preserving meat over time. However, there is ever increasing evidence that such additives may have harmful, even carcinogenic drawbacks. These drawbacks detract from the use of these compounds as mechanisms for the long term preservation of meat.

With the introduction of reliable means for refrigeration, i.e., the ability to maintain a 1.5 temperature regardless of the external environment, the long-term preservation of raw meat has been greatly enhanced and greatly increased the duration of the preservation. Frequently, in modern meat processing, animals are slaughtered at 20 one place which can be remote from the point of sale and the eventual consumer, and as much as a week can pass before the meat is actually consumed. This lag between the slaughtering of the meat and consumption requires that the meat 25 constantly maintained under refrigeration in order

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to preserve its quality and prevent its degradation over this time period.

For example, an animal (a cow) may be slaughtered and cut into halves or quarters which are then forwarded to a wholesaler or retailer where they may be divided into smaller cuts such as steaks or roasts. During the transfer of the meat from the slaughter house to the wholesaler or retailer, the meat must be maintained, frequently the meat is frozen in order to preserve its quality. After the meat has been divided into cuts for sale to the eventual consumer, it must also be maintained under constant refrigeration in order to preserve its quality. Under this distribution scheme, it can be from a few days to more than a week before the meat is purchased and consumed. It, therefore, becomes evident that this constant requirement for very low temperatures greatly contributes to the cost of meat.

20 Another example of the costly disadvantages of very low transportation and storage temperatures can be illustrated by practice of long distance overseas shipment and distribution of frozen meat. Today, freezing is a standard 25 method of distributing meat processed in one region of the world to another region where it is to be

consumed. Overseas shipment of frozen meat is both very costly and thawed meat obtained by this method is no longer considered to be "fresh" meat. That is, once a piece of meat has been frozen, by definition it is no longer considered to be "fresh." A method of overseas transportation of meat which maintains the "freshness" of meat transported for distribution would be highly desirable. Since the only method available for long distance overseas distribution of meat is by shipping frozen meat, no method currently exists which would allow for the overseas distribution of "fresh," unfrozen meat.

Transportation of slaughtered meat from

the slaughter house to the wholesaler or retailer
requires the use of some form of refrigerated
transportation, such as refrigerated tractortrailer trucks. This is a costly mode of
transportation since it requires specialized
equipment and extra fuel to provide and maintain
refrigeration.

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In addition to preserving the overall quality and fitness of the meat for consumption, other methods have been derived which are aimed at preserving the color of fresh meat. That is, methods have been developed which maintained for example, the red color of fresh meat, such as beef.

Typical examples of methods for treating raw meat to preserve the color of the meat are disclosed in United States Patent Nos. 3,459,117 to Koch et al., 4,001,446 and 4,089,983 both to Hood, and 4,522,835 and 3,930,040 to Woodruff et al. All of these patents disclose methods or processes for preserving or maintaining the color of meat such as beef, poultry or fish.

Both of the Hood references disclose methods of exposing an animal protein source to a reducing agent and then an environment of carbon monoxide in order to preserve the bright red color of protein source. Additionally, the Hood et al. references only treat slurries of the protein source as this is required for saturation by the carbon monoxide. The source is then mixed with the remainder of the food stuff to prepare a moist dog food. Further, the references are concerned only with the application of carbon monoxide in order to preserve the color of product and both require

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subsequent processing, such as canning or heat sterilization, in order to preserve the actual quality and freshness of the product.

Additionally, the Hood '983 reference discloses the addition of a sufficient amount of microbiological and bacteriological inhibitors to further preserve the product.

The Woodruff et al. '835 reference discloses a process for maintaining a good color and the freshness meat by first exposing meat to an atmosphere with a small amount of oxygen and then exposing the meat to a modified atmosphere containing a small amount of carbon monoxide to effect the conversion of myoglobin to carboxymyoglobin. A third required step is the maintenance of the meat in an atmosphere of higher than 10% carbon dioxide.

The Woodruff et al. '040 patent discloses a process for storing or shipping fresh meat in a 20 modified gaseous atmosphere. The process requires maintaining refrigerated meat in an artificial atmosphere composed of oxygen, carbon dioxide and carbon monoxide as well as nitrogen. The carbon monoxide may be removed from the modified material 25 after the meat has been treated for at least one hour.

Woodruff et al. patents maintaining the color in meat by treating the meat with a mixture of gases including carbon monoxide. That is, the Woodruff et al. patents teach chemical alteration of the surface of the meat to maintain the color of the meat and utilize refrigeration for meat preservation. Additionally, the Woodruff et al. patents teach the treatment of meat using a gaseous mixture of carbon monoxide, oxygen, carbon dioxide, and nitrogen. This method of treatment 10 results in the creation of a storage environment which has low oxygen concentration and a carbon dioxide concentration of approximately ten percent. This type of gaseous mixture creates optimal growth conditions for the growth of microaerophil bacteria 15 such as Helicobacter pylori and Campylobacter jejuni which are known to be pathogens which cause widespread gastroenteritis. The Woodruff et al. method of treating meat does maintain the color of fresh meat, however, the Woodruff et al. method has 2.0 the disadvantage of accelerating bacterial contamination of meat treated by the Woodruff et al. method, thus shortening the storage life of the meat treated thereby.

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The Koch et al. '117 patent discloses a cover useful for treating fresh red meat with carbon monoxide in order to maintain the bright red color of the meat. Koch et al., teaches a cover comprised of two films which are sealed together around the edges and which confines a quantity of carbon monoxide gas therebetween. Both film layers are substantially carbon monoxide impermeable when dry, however; when the film is brought into contact with a freshly cut sample of red meat, the moisture in the meat wets the film and transforms the film into a carbon monoxide permeable structure. The carbon monoxide then contacts the meat sample thereby causing the meat to maintain its desired red color.

The Australian Patent Document No.

AU-A-18559/92 to Tamayama et al., discloses a method for maintaining and improving the quality of meat by causing meat to contact and absorb carbon monoxide gas in a sealed container and then requiring removal of the carbon monoxide gas from the container. Exemplifying the criticality of the removal of the carbon monoxide gas from the container, the patent requires that the carbon monoxide gas within the container be sucked and discharge by means of a pump.

Heretofore, the treatment of raw meat with carbon monoxide has been taught simply as a mechanism for preserving the color of the meat and, not as a mechanism for the long-term preservation of a meat sample over time in a fresh, non-frozen form.

While the above-disclosed patents teach the exposure of raw meat to gas mixtures containing carbon monoxide or the exposure of meat slurries to carbon monoxide in combination with other steps, they fail to teach a simple method of exposing raw meat solely to carbon monoxide.

In order to overcome the problems and deficiencies of the prior art methods, it is desirable that a method of preserving raw meat be introduced which eliminates the cost and associated problems with the prior art preservation techniques.

Applicant has developed a single step
20 method for preserving meat by exposing raw meat to
an atmosphere consisting essentially of carbon
monoxide and, then, storing the meat in a sealed
container. Unlike prior art preservation methods,
no additional steps, compounds or additives are
25 required in order to prevent the growth of
microbiological or bacterial organisms.

### SUMMARY OF THE INVENTION AND ADVANTAGES

According to the present invention, a method for preserving meat by exposing raw meat to an atmosphere consisting essentially of carbon monoxide is shown. Meat treated according to the present invention may not require any form of subsequent refrigeration under certain conditions and time constraints and can be stored for long periods of time following treatment with the carbon monoxide without significant bacterial growth, without freezing, and without a loss in meat quality.

### BRIEF DESCRIPTION OF THE DRAWINGS

Other advantages of the present invention will be readily appreciated as the same becomes better understood by reference to the following detailed description when considered in connection with the accompanying drawings wherein:

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FIGURE 1 is a bar graph of the relationship between aerobic bacterial growth on a fresh meat sample stored at 22-30°C over time in either a CO treated environment or an air only environment;

FIGURE 2a is a histogram illustrating preservation duration of CO preserved meats and air treated meat preserved at 5  $\pm$  -/- 3°C as determined by Microaerophil growth;

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FIGURE 2b is a histogram illustrating preservation duration of CO preserved meats and air treated meat preserved at 5  $\pm$  -/- 3°C as determined by total viable aerobic bacterial growth;

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FIGURE 3 is a graph illustrating spectral analysis of the amounts of hemoglobin in the blood of cats that consumed either CO treated meat or air treated meat;

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FIGURE 4 is a photograph illustrating meats, the colors of meat treated with (A) vacuum only, (B)  $N_2$ , (C) air, and (D)  $CO_7$ 

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FIGURE 5 is a photograph illustrating the color change in meat treated without CO (left) and meat treated with CO (right);

FIGURE 6 is a photograph illustrating the
internal color change of meat treated without CO
(left) and meat treated with CO (right);

FIGURE 7 is a photograph illustrating the color change of a piece of fresh CO treated meat stored at  $5\,^{\circ}\text{C}$  for three days:

5 FIGURE 8 is a photograph illustrating the same meat sample shown in Figure 7 stored with CO at 5°C for ten days;

FIGURE 9 is a photograph illustrating a

10 transverse cut of the meat sample shown in FIGURE 8

made at 7 cm from the edge showing homogenous

bright red color;

FIGURE 10 is a photograph illustrating

transverse cuts of CO treated (left) and frozen
(right) meat samples after twelve days of storage;

FIGURE 11 is a photograph of the transverse cuts of meat shown in FIGURE 10 after 20 cooking;

FIGURE 12 is a photograph illustrating transverse cuts of the cooked meat samples shown in FIGURE 11; and

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FIGURE 13 is a photograph illustrating a section of CO treated meat as shown in FIGURE 10. following exposure to open air at 5°C for two weeks, at the end of this two week period, the meat sample was ground, a 200 gram "hamburger-like" sample was cooked and released CO was measured, (top) prior to cooking, (bottom) following cooking.

### DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

Generally, the present invention provides a method for preserving meat by exposing raw meat, processed or not, to an atmosphere consisting essentially of carbon monoxide (CO) subsequently, storing the meat in a sealed container.

the purposes of the present invention, the term "meat" is defined to include all types of fresh meat and fresh poultry such as beef, pork, veal, lamb, chicken, turkey, fish and 20 the like. The meat may be in the form of carcasses, primals (e.g., quarters), subprimals (e.g., top round), or retail cuts (e.g., steaks, ground meat and roasts). The process is also effective on whole animals including, but not limited to, cattle, chickens, and fish. prior art methods, the meat need not be slurried or

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otherwise pretreated. "Fresh meat" is defined as a meat article which has not been frozen and subsequently thawed before its sale or consumption.

By preserving, it is meant that the meat maintains a pleasing color, does not spoil and develop a foul smell, bacterial growth significantly inhibited or retarded, and remains completely pleasing, edible and consumable by humans and other animals. Preservation is not only maintained on the surface of the meat, but also throughout the entirety of the meat. That is, the meat is preserved throughout the thickness of the meat. "Pleasing color" implies that the color of the meat, preserved by the method according to the present invention, is such that it stimulates the appetite to consume the meat. That is, the color and odor of the preserved meat is such that a consumer would be enticed by the meat and would want to consume the meat. Again, meat color is also preserved throughout the thickness of the meat.

The term "without freezing" is defined as storing the meat wherein the temperature is kept between approximately -2 to 30°C. The term "without freezing" also excludes the use of any device or method for freezing the meat. Such

devices include, mechanical or electrical refrigeration devices such as refrigerators, freezers, coolers, and chillers. This term also excludes the preservation of meat by freezing through storage on ice.

Exposing raw meat to an atmosphere consisting essentially of carbon monoxide is defined as bringing into intimate contact both carbon monoxide gas and the meat being treated. The atmosphere preferably consists of carbon monoxide. This term also includes the complete conversion of myoglobin present in the meat sample to carboxymyoglobin and, the complete conversion of myoglobin to carboxymyoglobin/carboxyhemoglobin in fish. The meat is completely immersed or saturated with carbon monoxide.

More specifically, a cross-section of meat is completely immersed in or saturated to its core with carbon monoxide from the exposed surfaces through the entire cross-section (thickness) including its core region and retains the carbon monoxide until the meat is cooked. Thus, as stated above, the meat is preserved throughout its thickness.

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Carbon monoxide is inherently a very Carbon monoxide is relatively more inert gas. inert than nitric oxide gas (NO) released from nitrites which have been used as preservatives for meat for several hundred years. Carbon monoxide is a normal metabolite in the body. It is produced indigenously as a product of heme catabolism (mostly the breakdown of hemoglobin). Carbon monoxide is further converted to carbon dioxide and is released from the body in that form. Recently, it has been found that normal metabolism utilizes carbon monoxide as a neurological messenger. The high toxicity of carbon (Baranaga, 1993) monoxide generally stems from its ability to compete with oxygen for binding to hemoglobin.

Practically all of the carbon monoxide (over 99.9%) taken up by meat will be maintained as hemoglobin and myoglobin (Hb/Mb) bound forms. The distribution of carbon monoxide in the meat is assumed to be about half in each globin type. This estimation is based on the fact that mammalian muscles contain approximately two-thirds of their globins as hemoglobin and one-third as myoglobin, but when muscle becomes packed as meat, it looses a portion of its hemoglobin.

Both hemoglobin and myoglobin bind carbon monoxide much more strongly than oxygen. Native Hb/Mb contain iron and divalent oxidation state  $(Fe^{+2})$  and only in this form are Hb/Mb capable of binding the gas ligands  $O_2$ , NO, and CO. Following any change in the iron oxidation state, Hb/Mb loose their CO binding ability. Denaturation of the proteins (e.g. by heat) can also result in loss of CO binding potential (as well as other ligands).

Hb/Mb are established catalyzers of the oxidation process in biological tissues. Under regular atmospheric conditions, the Hb/Mb in fresh meat, which are in their native form, exist in a  $O_2$  bound form, the so-called oxy-Hb/Mb. Oxy-Hb/Mb tends to undergo autooxidation to met-Hb/Mb namely the oxidation of the Hb/Mb divalent iron to Fe<sup>+3</sup> can concomitantly with the formation of superoxide anion  $O_2^-$  by the reaction

oxy-Hb/Mb (
$$Fe^{+2}$$
... $O_2$ )  $\rightarrow$  Met Hb( $Fe^{+3}$ ) +

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The superoxide anion is unstable and further forms hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) which together with Hb/Mb acts as a highly active peroxidation system. Met-Hb/Mb no longer binds any 25 of the gas ligands including carbon monoxide. On the other hand, the Met-Hb/Mb are catalyzers of

oxidations. Unlike the case of oxygen bound to Hb/Mb, in a carbon monoxide bound form, Hb/Mb are protected from autooxidation. Therefore, to protect meats from autooxidation, carbon monoxide is best applied to fresh meat.

It is thought that the mechanism for carbon monoxide preserving of meat is the much greater affinity of myoglobin for carbon monoxide than for oxygen. Following this mechanism, carbon monoxide out-competes oxygen for binding onto myoglobin molecules within the meat structure. completely displacing oxygen, the micro-environment of the meat becomes more anaerobic and, thereby, growth of aerobic prevents or inhibits the microorganisms, such as Escherichia coli, which are responsible for spoilage and degradation of fresh meat and illness. Anaerobic bacterial growth, such as Microaerophils, is also inhibited when this method is utilized. This proposed mechanism of carbon monoxide action is merely for illustrative purposes and in no way should be construed as limiting.

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The ability to inhibit or prevent the growth of microorganisms allows for the extended storage of meat treated according to the present method. That is, meats treated according to the present invention have a longer storage life and remain both viable and edible in a non-contaminated form for periods longer than those available using current preservation techniques.

In the practice of the present invention,

10 meat samples are placed in an enclosure or
container and flushed or exposed to carbon monoxide

gas.

The process consists of two stages:

- (A) "Meat packing" which refers to introducing the meat into a confined CO atmosphere.

  "Packed meat" refers to meat which has undergone the meat packing part of the process.
- (B) "Meat preservation" which involves 20 maintaining the "packed meat" until it reaches the consumer.

### Meat Packing

The container for treatment, storage, and transportation of meat by the method of the present invention can be constructed of various gasimpermeable material such as plastic, metal, and

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other materials known in the art. The container can be equipped with both gas inlet and outlet channels which can be opened to allow the influx of gas (CO) or closed in order to render the container sealed.

A suitable container would be capable of maintaining a seal to prevent the escape of carbon monoxide gas from the container. For example, the container can be a sealed room in which large amounts of meat may be treated at a given time, the container can also be a smaller sealable container or chamber. Preferably, the container is of larger volume than the volume of meat being treated to allow for a greater volume of carbon monoxide gas to contact the meat sample.

In a preferred embodiment of the invention, meat samples are treated and stored within plastic bags constructed of a material which is safe for the storage of food products such as polyvinylidene chloride. Preferably, the plastic bags will be constructed of a material that is impermeable to the passage of gases therethrough. Thusly, the meat is maintained in the carbon monoxide atmosphere within the bag (container) during storage.

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After a piece of meat to be treated according to the method of the present invention is placed in a suitable container, the container is then filled with the CO gas. The addition of the CO gas can be accomplished in any suitable manner; however, the preferred methods include first removing the gas atmosphere present in the container (usually air) by using a vacuum pump, as is well known in the art, to remove any gases present and the container. The container is then filled with CO from a source such as a gas cylinder.

The container is connected to the CO containing cylinder and CO is introduced. and output pressures are measured during the filling process. The input pressure is generally maintained within a range of approximately 1.5 to 5.0 atmospheres. The preferred pressure is approximately 2.0 atmospheres. Upon reaching the preferred pressure in the output, the gas flow is stopped and excess gas is allowed to escape until pressure within the the container reaches approximately 1.0 to 1.2 atmospheres. The preferred gas pressure in the container is approximately 1.1 atmospheres.

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During the gas filling operation, the ambient temperature of the surrounding can be maintained between -2 to 37°C.

The parameters that govern gas filling or exposure time vary depending on the pressure of the gas input, the dimensions of the inlet and outlet channels, and the dimensions of the container.

For meat packing, exposure of only the surfaces of the meat to carbon monoxide is generally required. However, for the purposes of meat preservation, the gas filling time should be long enough to allow for a sufficient amount of CO gas to be completely absorbed (throughout its thickness) into the meat undergoing treatment. That is, enough CO gas is flushed through the container to allow for the complete penetration and protection of the meat being treated.

The gas filling time generally ranges from approximately one to thirty minutes with the preferred filling time being approximately five minutes. For the purposes of this invention, exposure time is defined as the gas filling time. Again, it should be noted that the length of exposure of the carbon monoxide to a meat sample will vary depending on the size of the meat sample and the weight of the meat sample being treated.

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That is, a larger and heavier meat sample will require a longer period of exposure to the carbon monoxide in order to achieve long-term preservation. In other words, a larger meat sample will require a longer exposure to carbon monoxide in order to properly preserve the meat sample without the freezing.

The temperature during the carbon monoxide exposure is preferably between -2 and 37°C and can vary depending on the temperature selected to in order to carry out the method.

Meat treated as previously described above generally contains from 5 to 100% by weight or volume of CO gas. The preferred volume of CO in the treated meat is approximately 30% of the weight of the meat (e.g. 30 ml for 100 grams of treated meat).

Under the meat preservation method of the present invention, the meat surface is initially contacted with the CO gas. Since the surface of the meat is the most prominent site for the presence of bacteria, the meat treated by the method of the present invention is immediately protected. Further, while sealed in the container, penetration of the CO gas continues until the entire meat mass has been penetrated and, thereby,

protected. This total penetration allows for the complete substitution of both hemoglobin and myoglobin by the carboxy forms of these compounds as is shown in the following examples. The total CO treatment of the meat throughout its thickness also enables meat which has been treated according to the present invention to maintain a pleasing color for extended periods of time after the meat has been removed from the packaging or container in which it was treated. That is, as shown in the following examples, meat treated according to the present invention can be transported, unpacked, and then maintained in a fresh form for a further extended period of time without a loss of color or quality.

The above discussion provides a factual basis for the use of the present invention as a method of long-term preservation of meat at different temperatures without freezing. The examples also demonstrate the preservation of the meat after undergoing the treatment of the present invention. The methods used with and the utility of the present invention can be shown by the following examples.

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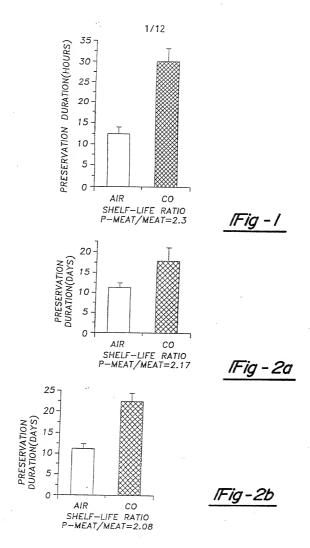
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## A METHOD FOR THE LONG-TERM PRESERVATION OF MEAT AND THE MEAT PROCESSED THEREBY

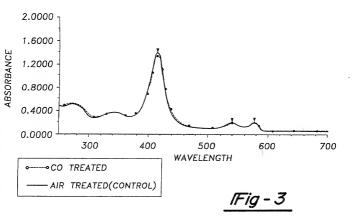
#### ABSTRACT OF THE DISCLOSURE

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A method for preserving meat and the meat processed thereby is disclosed. The method includes the steps of exposing raw meat to an atmosphere consisting essentially of carbon monoxide and maintaining the meat in a sealed container to maintain color and freshness while retarding bacterial growth.







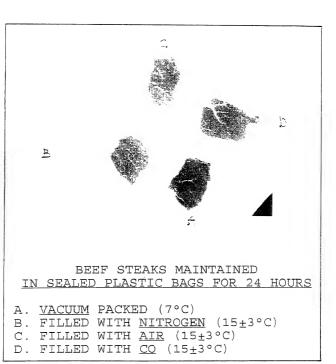


Fig - 4

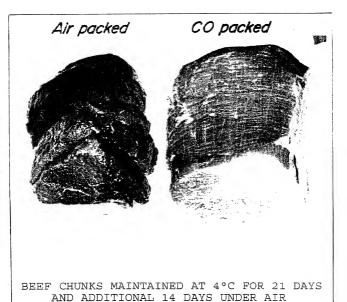


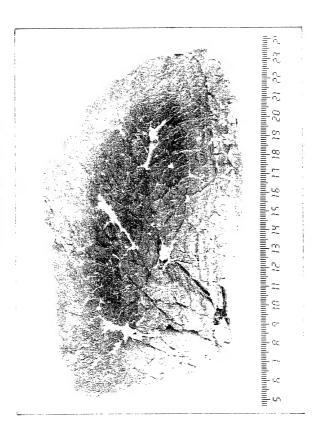
Fig-5



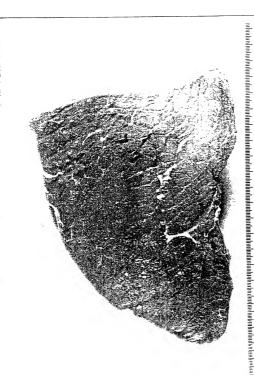
### BEEF CHUNKS MAINTAINED AT 4°C FOR 21 DAYS

RIGHT: CO PACKED LEFT: AIR PACKED

IFig-6



Trig-7



8 9 16 11 12 13 14 15 16 11 18 19 20 21 2 Fig - 8



ted of  $(d^2, (d^2, (d^$ 

Fig - 9

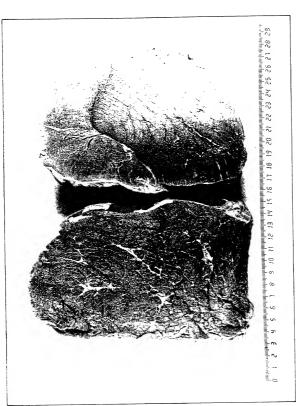
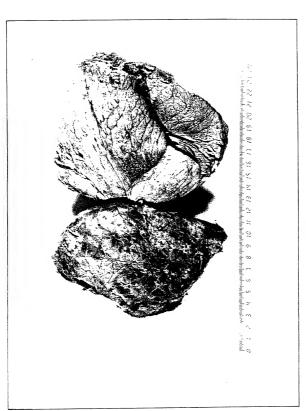


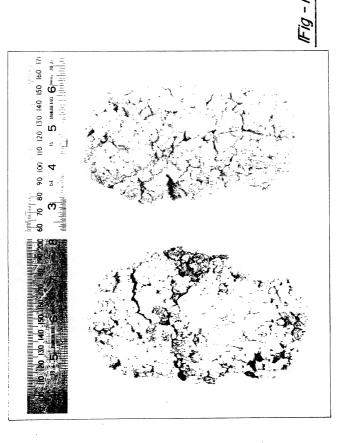
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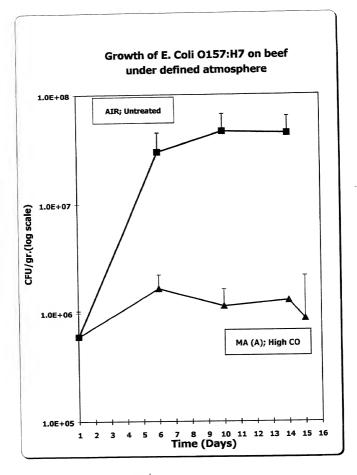


IFig - 11



Fig - 12





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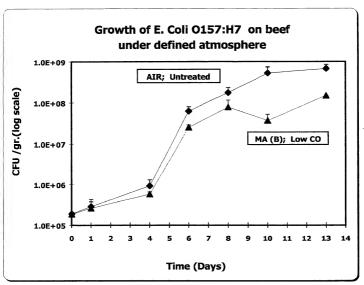
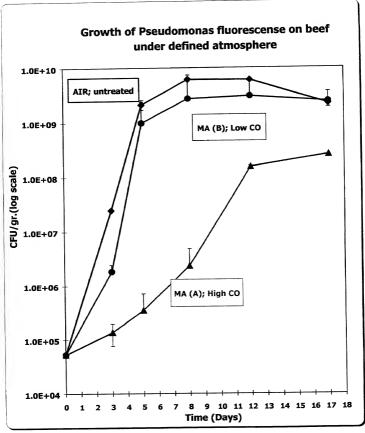


Fig 15



F15 16

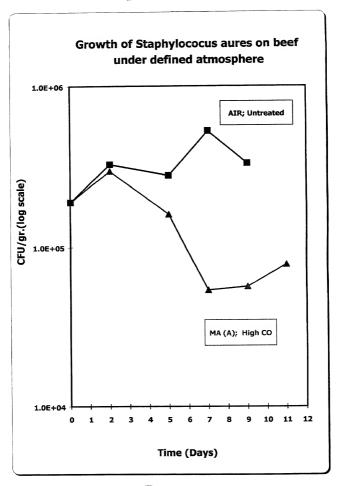


Fig 17

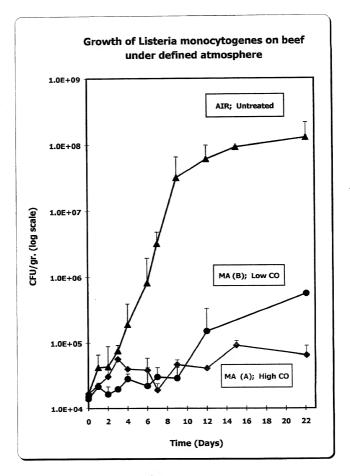


Fig 18

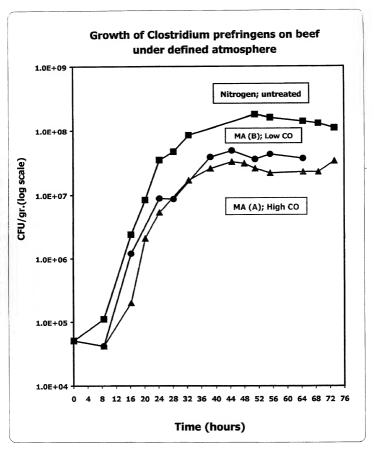


Fig 19

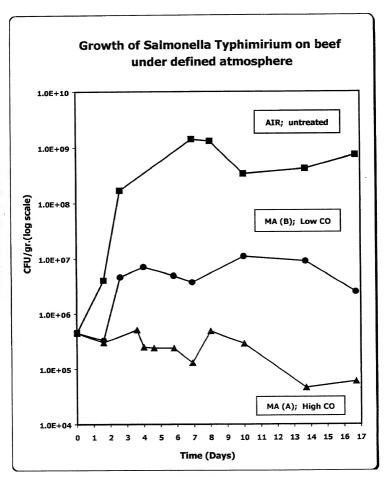
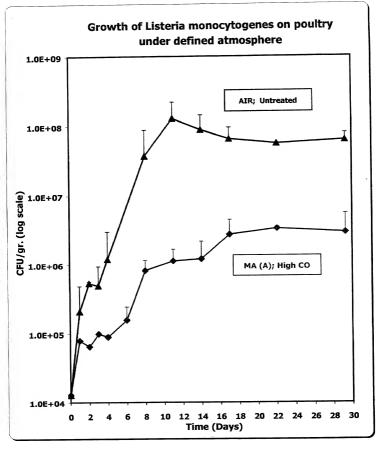
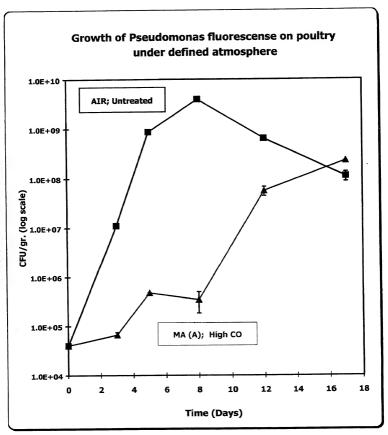
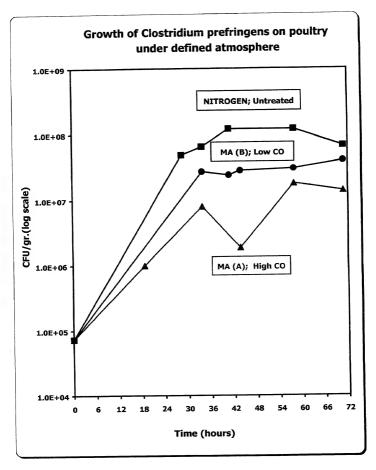


Fig 20





F1922



F1923

#### COMBINED DECLARATION AND POWER OF ATTORNEY

(ORIGINAL, DESIGN, NATIONAL STAGE OF PCT, SUPPLEMENTAL,
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As a below named inventor, I hereby declare that:

#### TYPE OF DECLARATION

This declaration	is of the following type: (check one applicable item below)
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NOTE:	If the declaration is for an international Application being filed as a divisional, continuation or continuation-in-part application do not check next item; check appropriate one of last three items.
national st	age of PCT
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X continuati	on-in-part (CIP)
	INVENTORSHIP IDENTIFICATION
WARNING:	If the inventors are each not the inventors of all the claims an explanation of the facts, including the ownership of all the claims at the time the last claimed invention was made, should be submitted.
My residence, po- first and sole inve	st office address and citizenship are as stated below next to my name, I believe I am the original, intor (if only one name is Itsted below) or an original, first and joint inventor (if plural names are

# listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled: A METHOD FOR THE LONG-TERM PRESERVATION OF MEAT AND THE

# MEAT PROCESSED THEREBY SPECIFICATION IDENTIFICATION

the specification of which: (complete (a), (b) or (c))	
(a) x is attached hereto.	
(b) _ was filed on as _ Serial No. 08/ or	
Express Mail No., as Serial No. not yet known and was amended on	(if applicable

NOTE: Amendments filed after the original papers are deposited with the PTO which contain new matter are not accorded a filing date by being referred to in the declaration. Accordingly, the amendments involved are those filed with the application papers or, in the case of a supplemental declaration, are those amendments claiming matter not encompassed in the original statement of invention or claims. See 37 CPR 1.67.

(Declaration and Power of Attorney [1-1] page 1 of 4)

(c) x was described and claimed in PCT International Application No. PCT/US96/05373 filed on October 15, 1997 and as amended under PCT Article 19 on NONE (if any).

#### ACKNOWLEDGMENT OF REVIEW OF PAPERS AND DUTY OF CANDOR

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations. § 1.56(a).

\_ In compliance with this duty there is attached an information disclosure statement 37 CFR 1.97.

#### PRIORITY CLAIM

I hereby claim foreign priority benefits under Title 53, United States Code, § 119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed.

(complete (d) or (e))

(d) X no such applications have been filed.

(e) \_\_\_ such applications have been filed as follows

NOTE: Where item (c) is entered above and the International Application which designated the U.S. claimed priority check item (e), enter the details below and make the priority claim.

#### EARLIEST FOREIGN APPLICATION(S), IF ANY FILED WITHIN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO THIS U.S. APPLICATION

COUNTRY	APPLICATION NUMBER DATE OF FILING PRIORITY CLAIM (day, month, year) UNDER 35 USC 119	
	_YES _NO	
	_YES _NO	
	_YES _NO	
	YESNO	
	_YES _NO	

## ALL FOREIGN APPLICATION(S), IF ANY FILED MORE THAN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO THIS U.S. APPLICATION

PCT/US96/05373; filed 4-18-96, priority 4-19-95.

(Declaration and Power of Attorney [1-1] page 2 of 4)

# TOTAL CONTROL

#### POWER OF ATTORNEY

As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith. (List name and registration number)

Kenneth I. Kohn

Reg. No. 30,955

Ilene N. Montgomery Reg. No. 38,972

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DIRECT TELEPHONE CALLS TO:

(NAME AND TELEPHONE NUMBER)

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#### DECLARATION

I hereby declare that all statements made herein of my own knowledge are true and that all statements made information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

#### SIGNATURE(S)

~	
Full name of sole or first inventor	Nurith Shaklai
Full name of sole or first inventor Inventor's signature	Den
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Inventor's signature	
Date Country of Citizenship	
Residence	-
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-	Signature for inventor who refuses to sign or cannot be reached by person authorized under 37 CFR 1.47. Number of pages added $\_$				
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-	Added pages to combined declaration and power of attorney for divisional, continuation, or continuation-in-part (CIP) application.				
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(Declaration and Power of Attorney [1-1] page 4 of 4)

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